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Prevalence of Zidovudine Induced Megaloblastic Anemia among Human Immunodeficiency Virus Positive Patients Attending University of Gondar Hospital Antiretroviral Therapy Clinic, Northwest Ethiopia

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Abstract

Background: The use of antiretroviral treatment containing Zidovudine in HIV infected patients is associated with hematological toxicity to varying degrees of cytopenia particularly megaloblastic anemia. However, there is scarcity of data which assesses prevalence of AZT induced megaloblastic anemia among HIV patients taking ART in Ethiopia.

Objective: The aim of this study was to assess the prevalence of Zidovudine induced megaloblastic anemia in HIV/AIDS patients taking ART at University of Gondar Hospital, Northwest Ethiopia.

Method: A retrospective study was conducted among HIV/AIDS patients who were attending Gondar University Hospital between January 2012 and February 2013. Data was collected from 319 patients' chart and statistical analysis was done on SPSS version 20.

Result: Prevalence of AZT induced anemia was 11.3% at the 6 month of AZT based treatment. Most of them (n = 29, 80.6%), developed macrocytic anemia. Age shows significant association with the incidence of anemia (P = 0.042) while sex, baseline CD4 cell count, presence of opportunistic infection, HIV clinical stage and baseline hemoglobin were not associated.

Conclusion: This study showed high prevalence of macrocytic anemia after 6th months of treatment with AZT based ART.

Keywords: Megaloblastic Anemia, Antiretroviral Therapy, Zidovudine

Abbreviations: **AIDS**: Acquired Immune Deficiency Syndrome; **ART**: Antiretroviral Treatment; **AZT**: Zidovudine; **CBC:** Complete Blood Count; $CD4^+$: Cluster Differentiation 4; **CDC:** Center for Disease Control; **FMOH:** Federal Ministry of Health; **HAART:** Highly Active Antiretroviral Treatment; **HIV:** Human Immunodeficiency Virus; **MCH:** Mean Cell Haemoglobin; **MCHC:** Mean Cell Haemoglobin Concentration; **MCV:** Mean Cell Haemoglobin; **MOH:** Ministry of Health; **OI:** Opportunistic Infections; **RDW:** Red Cell Distribution Width; **WHO:** World Health Organization

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Background

HIV infection is frequently associated with hematological abnormalities such as anemia which is increasing in frequency and severity with progression of infection to immunologic and clinical AIDS [1]. Typically the anemia may result from low production of red blood cells, increased RBC destruction, or ineffective RBC production and frequently the laboratory features are compatible with anemia of chronic disease with a low reticulocyte count, normocytic normochromic red blood cells with normal iron stores and cytokine mediated poor erythropoietin response [2, 3].

Highly active antiretroviral therapy (HAART) is used to control the reproduction of the virus and slow or halt the progression of HIV-related diseases. The introduction of HAART in 1996 with three antiretroviral (ARV) drugs primarily in the combination of one Protease Inhibitor (PI) and two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) has led to significant reduction in AIDS-related morbidity and mortality [4]. Now that access to HAART is hopefully increasing, AZT also increasingly prescribed in settings with limited laboratory facilities compared with industrialized countries. Because generic antiretroviral therapy has been shown to effectively work in resource-limited settings selectivity of the drug is better than other regimen. AZT is a well-known cause of drug-induced hematotoxicity [5].

Although different studies suggested HIV-related hematological complication improved with HAART regimen treatment including the maintenances in functional status, energy levels, fatigue and overall improvement in quality of life [6], HIV-positive patients receiving highly active antiretroviral therapy (HAART) still develop mild to moderate anemia and associated quality of life impairment [7]. Especially ART regimen containing Zidovudine are associated with anemia with bone marrow suppression, where the suppression appears to be more common in those patients with advanced diseases, related to dose and duration of the treatment [8]. Drug-related anemia was positively associated with prescription of AZT. AZT is the commonest cause of drug associated anemia in which patients taking AZT develop anemia after 3 months of first initiation and consequently changing the regimen other than Zidovudine due to suppression of bone marrow [9] and induces megaloblastic anemia [10]. AZT induced Megaloblastic anemia is caused whenever the value of MCV is >100fl during the treatment and declined at a time of AZT changed to other regimen [11].

Even if, the causes of anemia have different factors, Zidovudine induced megaloblastic anemia also has taken places its quotient at large. The research conducted on the prevalence of Zidovudine induced anemia in HIV patients which are conducted at different countries of the world showed the prevalence ranging from 1.3% to 95% [9]. However, most of the studies conducted on HAART/AZT induced anemia focus on general anemia. Thus, this study was conducted to assess the prevalence of Zidovudine induced megaloblastic anemia in HIV patients attending University of Gondar Hospital, Northwest Ethiopia. It provides information on the prevalence of megaloblastic anemia associated with Zidovudine treatment and provides information for concerned bodies to develop appropriate measure of reducing the risk.

Methods

Study setting and population

A hospital based retrospective study was conducted on 319 HIV positive patients who were taking AZT based ART regimen and had a follow-up at University of Gondar Hospital ART clinic between January 2012 and February 2013. A patient who had lost follow-up and did not have full information was excluded from this study. Also pregnant women, patients with chronic kidney disease, rheumatic arthritis, cancer patients and gastrointestinal diseases were excluded from the study. Data collection sheet was used to collect the data from the patients' record book. It includes initiation date of Zidovudine regimen with hematological parameters at baseline, at 6^{th} months and at 12^{th} months. To ensure the completeness, accuracy and consistency of information during data collection, cross check before cleaning and entering the data was done.

Statistical analysis

The data obtained was coded and entered into SPSS version 20 statistical software. Cleaning was done before the analysis of

the data. Descriptive statistics was used to get clear picture of dependent and independent variables at baseline, 6th month and 12th month. To determine association of anemia incidence with age, sex, baseline CD4 cell count, baseline hemoglobin and WHO clinical stage chi-square test was performed. P <0.05 was considered as significant.

Ethical consideration

Ethical clearance was obtained from of School of Biomedical and Laboratory Sciences Ethical Committee, College of Medicine and Health Sciences, University of Gondar before commencement of study. A formal letter of permission was submitted to University of Gondar Hospital ART Clinic and approved in order to conduct the research in the study sites. Confidentiality was kept by recoding the data with codes and unauthorized person didn't have access to the data.

Results

A total of 319 patient charts were reviewed from ART clinic of University of Gondar Hospital. Of these, majority 202 (63.3%) were females and the mean age was 34.3±8.2 years, with age ranging from 19 to 65 years. About 255 (79.9%) of them were in the age range 25 - 44 years. Around half 162 (50.8%) of the patients were on WHO AIDS clinical stage III and 19 (6.0%) of patients were having opportunistic infection (tuberculosis). Nearly half of the patients' baseline CD4 cell count was >200 cells/µl of blood. After 6th months of AZT exposure about 160 (50.2%) of the total study participants switched to non AZT based therapy (Table 1).

Table 1: Characteristics of patients receiving antiretroviral therapy at ART clinic in University of Gondar Hospital, January 2012 to February 2013

Characteristics		Frequency	Percentage
Sex	Male	117	36.7
	Female	202	63.3
Age in years	15-24	26	8.2
	25-44	255	79.9
	45-64	37	11.6
	>64	1	0.3
WHO AIDS clinical stage	I	31	9.7
	II	101	31.7
	III	162	50.8
	IV	25	7.8
Opportunistic infection	Yes	19	6.0
	No	300	94
Baseline CD4 cell/µl	<200	162	50.8
	>200	157	49.2
Regimen Change	TDF+3TC+EFV	146	45.8
	d4T+3TC+NVP	14	4.4

The prevalence of AZT induced anemia among study population was 36 (11.3%) at the 6th month of AZT treatment. Of these, majority 18 (50%) of them showed mild type of anemia. At the 6th month of initiation of AZT, 29 (80.6%) of the patients developed macrocytic anemia (MCV>100) which may reach to 131fl and most 13 (36.1%) of the patients average hemoglobin concentration of red cells was normochromic followed by hyperchromic red cells 12 (33.3%). The prevalence, severity of anemia and degree of red cell macrocytosis has declined from 6th month of AZT treatment to the 12th month (Table 2).

Table 2: Prevalence and characteristics of anemia among patients taking AZT based treatment at University of Gondar ART clinic from January 2012 to February 2013

Variable		Baseline n (%)	6 month n (%)	12 month n (%)
Anemia	Yes	6 (1.9)	36 (11.3)	3 (0.9%)
	No	313 (98.1)	283 (88.7)	316 (99.1)
Severity of anemia	Moderate: Hgb 8 -10 g/dl	3 (0.9)	18 (50)	2 (0.6)
	Severe: Hgb < 8 g/dl	3 (0.9)	18 (50)	1 (0.3)
MCV	Normocytic : 80-100fl	4 (66.7)	7 (19.4)	2 (66.7)
	Microcytic: <80fl	-	-	
	Macrocytic: >100fl	2 (33.3)	29 (80.6)	1 (33.3)
MCHC	Normochromic: (32-36%)	2 (33.3)	13 (36.1)	1 (33.3)
	Hypochromic : < 32%	4 (66.7)	11 (30.6)	2 (66.7)
	Hyperchromic: >36%	-	12 (33.3)	-

The association between incidence of anemia after taking AZT based treatment with factors like age, sex, baseline CD4 cell count, baseline hemoglobin, presence of opportunistic infections and WHO clinical stage was performed but there was no statistical significant association between the incidence of anemia with all the variables except age (P =0.04) (Table 3).

Table 3: Association between anemia and independent variables among HIV patients taking AZT based treatment University of Gondar Hospital ART clinic, January 2012 to February 2013

Variables		Anemia status		P- value
		Non anemic	Anemic	
Sex	Male	106	11	0.46
	Female	177	25	
Age in years	15-24	23	3	0.042
	25-44	224	31	
	45-64	36	1	
	>64	0	1	
Baseline CD4 Cell/µl	<200	143	19	0.86
	>200	140	17	
Opportunistic infection	Yes	15	4	0.24
	No	268	32	
WHO clinical stage	I	29	2	0.21
	II	91	10	
	III	144	18	
	IV	19	6	
Baseline hemoglobin	Non anemic	279	34	0.13
	Anemic	4	2	

Discussion

Since there is no specific guidance for AZT-induced anemia management, all antiretroviral clinics simply followed the National Guidelines, i.e. changing AZT to other antiretroviral drugs if Hgb < 6.5 g/l or in patients with severe anemia. The AZT induced anemia is rather unique and should be properly managed [12].

Even if the exact mechanism of the anemia is still unknown, it was hypothesized that AZT may suppress erythropoiesis or inhibit erythroid stem cells, thus causing pure red cell aplasia (i.e. decreased reticulocyte counts and Hgb levels without hemolysis or blood loss), increasing mean corpuscular volume (MCV) and elevating erythropoietin levels [5, 13]. In addition, AZT inhibits beta-globin gene expression and has bone marrow cytotoxicity [14]. In other hands, the experimental study conducted in Kentucky, USA hypothesized that AZT combination exposure causes immune cell populations in the bone marrow to undergo apoptotic cell death, and that the toxicity would affect the host response to an infectious stimulus and severe macrocytic anemia [15].

The prevalence of macrocytic (megaloblastic) anemia was higher 36 (11.3%) after initiation of AZT containing regimen at 6^{th} month than to baseline 6 (1.9%) and 12^{th} month 3 (0.9%). About 1.9% of patient was anemic at baseline and exacerbated to more anemia after taking AZT containing regimen. Subsequently after 6^{th} months of AZT exposure about 160 (50.4%) of the total study participants switched to non-AZT containing drug and become the least anemic after 12^{th} month of ART follow up. However, it seems imbalance of anemia between 6^{th} month and 12^{th} month clearly shows that AZT was

myelosuppressive drug due to significantly declined anemia successive to 6th month.

The finding of this study was less than study from Indian, which shown the prevalence AZT induced anemia of 16.2% which had occurred within 6th months of initiation of therapy and the peripheral smear showed macrocytic anemia in almost half of the patients and in the remaining showed normocytic and normochromic anemia [16]. The study is not in accordance with this study prevalence; the reason might be the difference of anemia grading that we had used from management of anemia for chronic diseases and HIV/AIDS [11].

The study conducted in Uganda, reflects severe anemia was prevalent at ART initiation and subsequently having a new tuberculosis and baseline anemia were not associated with AZT induced anemia [17]. From saying improvement we hypothesize that taking Zidovudine with other ARV drugs, can reduce the viral load to extremely low levels, and increase the CD4 cell counts to promote staying healthier longer. But megaloblastic anemia caused by AZT was without association of these factors. The study was in line with our result with regard of determinant factors shown that there were no significant association for AZT induced anemia with that of 6th month hemoglobin.

In other hand, the number of patients developed OI's was a few; but the majority of WHO AIDS clinical stage were at stage III, which OI's might developed next to stage IV according to WHO criteria. The reason of non-association of 6th month HGB with OI's and clinical stage was that, both OI's and WHO clinical stage data were collected for baseline. Because after treatment the previous stage might improves result of HAART and subsequently the staging system also depending treatment assigned as (T1 to T4).

The highest rate of anemia was registered for 6th month (11.3%) and this was in line with reports from Namibia 11.3% and Thailand 11.2% which is in concordant with our result. From our result prevalence (11.3%), 29 (80.6%) of them developed macrocytic anemia which means, >100fl reached to 131 fl. The reason for having a macrocytic type of anemia might be due to the compensatory red cell production and early circulation of immature hyperchromic cells.

One of the study conducted in Ivory Coast, revealed the prevalence of AZT induced anemia was 9.6%. It was somehow lower than the prevalence in this study. The reason for slightly higher prevalence of anemia in the current study might be due to the variation of grading of anemia.

Most of the prevalence of AZT induced anemia was closest to our study. The study conducted in Southern Ethiopia in Hawassa referral hospital also testifies this result; prevalence was 12.0% which was related to this study result 11.3%. The specification of this study revealed the particular AZT induced megaloblastic anemia could help physician for management of anemia accordingly. The prevalence was in accordance with all other studies conducted in many countries that reported 4.0 to 21.9% of anemic cases [13, 18]. Moreover, in some cases the anemia quickly developed; thus severe anemia was just detected; that was possibly due difference to patients' socio demographic and study design.

Conclusion

This study higher prevalence of anemia was observed after taking AZT based HAART for 6th months and most of anemic patients showed macrocytic blood picture with mild anemia. There was no statistical significant association between incidence of anemia and independent variables (sex, baseline HGB, baseline CD4 cell count, presence of OI and WHO clinical stage). Since the exact mechanism of AZT for causing anemia is not well elucidated, it will be good to conduct pharmacological studies for better monitoring and successful treatment.

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